U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-290 036

Interaction of Chemical Pollutants and Virus in a Crustacean: A Novel Bioassay System

(U.S.) Environmental Protection Agency, Gulf Breeze, FL

1977

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA-600/J-77-140	2. JOURNAL ARTICLE	3. RECIPIENT'S ACCESSION NO. PB290036
4. TITLE AND SUBTITLE INTERACTION OF CHEMICAL POLLUTANTS AND VIRUS IN A CRUSTACEAN: A NOVEL BIOASSAY SYSTEM		5. REPORT DATE 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) John A. Couch and Lee Courtney		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Same as box 12		10. PROGRAM ELEMENT NO. 1EA615 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory, Gulf Breeze Office of Research and Development U. S. Environmental Protection Agency Gulf Breeze, FL 32561		13. TYPE OF REPORT AND PERIOD COVERED Final 14. SPONSORING AGENCY CODE EPA/600/04

15. SUPPLEMENTARY NOTES
REFERENCE: ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, Volume 298, pages 497-504, 1977, 25360

16. ABSTRACT

Pathogen-host interactions were investigated with a simple "host-pathogen-interaction" system, using as tentative criteria of interaction: increase in viral prevalence, increase in infection intensity, increase in mortality, and enhanced cyptopathic effects in exposed-infected shrimp as compared to control-infected shrimp. A large group of shrimp, 23.3 percent of which had light patent Baculovirus infections, was divided equally into two groups. One group was exposed to the chemical stressor Aroclor 1254 at 0.7 parts per billion for 35 days in flowing seawater. The other group was maintained as control in flowing seawater. Viral prevalence in exposed shrimp samples increased with time at a significantly greater rate than in control shrimp. Viral prevalence in Aroclor-exposed shrimp survivors was 75 percent after 35 days, whereas only 45.7 percent of control shrimp had patent viral infections. This finding suggests an interaction among the chemical stressor (Aroclor 1254), host, and virus. Nature or mechanism of the interaction has not been defined, but the shrimp-virus system shows promise for future bioassays to determine influence of low concentrations of pollutants on natural pathogen-host interactions.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Criteria control Baculovirus Bioassay Pollutants	Pathogen-host interactions Exposed-infected shrimp Control-infected shrimp Chemical stressor Aroclor-exposed shrimp-virus system Aroclor 1254	08/A 06/F 06/C 06/T

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Unlimited	unclassified	11
	20. SECURITY CLASS (This page) unclassified	22. PRICE A02 A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/J-77-140
JOURNAL ARTICLE

PB 290036

INTERACTION OF CHEMICAL POLLUTANTS AND VIRUS IN A CRUSTACEAN: A NOVEL BIOASSAY SYSTEM

John A. Couch and Lee Courtney

Reprinted from
ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume 298 Pages 497–504
September 29, 1977 25360

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

11

INTERACTION OF CHEMICAL POLLUTANTS AND VIRUS IN A CRUSTACEAN: A NOVEL BIOASSAY SYSTEM*

John A. Couch and Lee Courtney

Gulf Breeze Environmental Research Laboratory
United States Environmental Protection Agency
Sabine Island, Gulf Breeze, Florida 32561

Introduction

Most established host-parasite or host-pathogen relationships are more or less balanced interactions of long evolutionary codevelopment. Any new environmental factor(s) extraneous to the host and parasite that would alter an established pattern in the host-parasite balance, either subtly or dramatically, might have significant long-range effects on the host-parasite relationship. In aquatic ecosystems, few experimental studies have been made on the possible interactions among such environmental factors as pollution, host species, and parasites or pathogens.[1] There are data available, however, that indicate an unusual adverse effect on some host aquatic species by certain natural parasites after or during exposure of the host-parasite system to specific pollutants or pollutant complexes.[2-4]

Recently, we have been investigating a newly discovered aquatic animal host-virus system in relation to its development as a bioassay tool for predicting possible interactions among chemical pollutants, host, and virus. The system that we have studied consists of penaeid shrimp as host, a shrimp-specific *Baculovirus*, and several selected pollutant chemicals, including polychlorinated biphenyls (PCBs).[5-8] PCBs were selected because of their widespread activity as aquatic pollutants and because of our knowledge of relative toxicity of PCB isomers to penaeid shrimps.[9] Concentrations of more that 3–5 parts per billion (ppb) of the PCB Aroclor® 1254 are very toxic to penaeid shrimp. Concentrations of less than 1 ppb of Aroclor 1254 are less acutely toxic.

In earlier experiments, we found that exposure of small groups of shrimp (eight to 35 per group), with a low natural prevalence of *Baculovirus*, to 1–3 ppb of PCB (Aroclor 1254) for 10–25 days resulted in an increase of the *Baculovirus* prevalence in some of the groups and, commonly, in increases in intensity of infection in exposed individuals.[8] These tests were repeated several times, and in two of three tests, the Aroclor 1254-exposed shrimp showed a higher prevalence and intensity of infection when compared to controls during and after exposure periods.[8]

Because of considerable variability in the above test results, however, we repeated the basic PCB exposure experiment with larger numbers of test and control shrimp. These experiments were repeated to determine whether PCB exposure of a group of shrimp, some of which were naturally infected with virus, was associated with an increase in virus prevalence and host mortality in the group.

*This report is publication 300 from Gulf Breeze Environmental Research Laboratory.

Materials and Methods

Three thousand live pink shrimp (juveniles to adults) were purchased from a commercial bait trawler at Keaton Beach, Florida. The shrimp were trawled from Apalachee Bay, Florida, a region that we have monitored for the shrimp virus for the past 5 years.[6,7] We have found that the pink shrimp there are infected. From 0 to 80% of any shrimp sample are infected; on the average, 5% of each sample is patently infected. The shrimp were placed in large flowing seawater tanks at the Gulf Breeze laboratory and acclimated to holding conditions for 1 week.

For the experiment, the surviving acclimated shrimp were divided into three large groups: an experimental group of 925, a control (nonexposed) group of 925, and a base sample of 150, from which we would attempt to determine the initial virus prevalence of the sample. The experimental and control groups were placed in separate round fiber glass tanks (2.4 m in diameter) that were supplied with flowing seawater from siphons that delivered a known volume of seawater per hour from an overhead reservoir water trough. Stand pipes in the tanks, set at the desired depth (0.5 m), delivered overflow from the experimental tank to a toxicant holding pond.

A commercial syringe apparatus was used to continuously inject a calculated volume of the PCB (Aroclor 1254) dissolved in triethylene glycol into the experimental tank water. The seawater siphon flow was 360 liter/hr for the experimental tank. Two syringe pumps each injected 0.354 μg/liter of Aroclor 1254 into the tank to maintain a nominal ambient concentration of 0.708 μg/liter of Aroclor 1254 continuously in the exposure tank. The exposure experiment lasted 35 days. During the exposure experiment, water and shrimp samples were taken weekly and analyzed by gas chromatography for actual concentrations of Aroclor 1254. Each week, 15

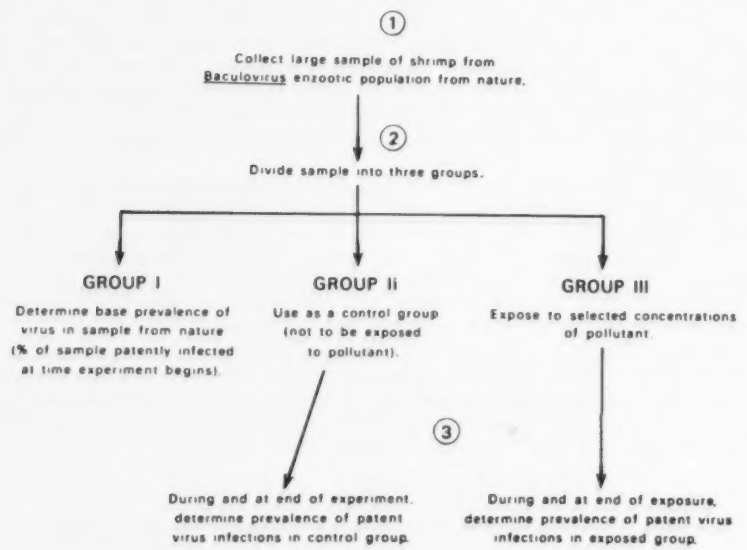
1
Collect large sample of shrimp from Baculovirus enzootic population from nature.
2
Divide sample into three groups.
GROUP I
Determine base prevalence of virus in sample from nature (% of sample patently infected at time experiment begins).
GROUP II
Use as a control group (not to be exposed to pollutant).
GROUP III
Expose to selected concentrations of pollutant.
3
During and at end of experiment, determine prevalence of patent virus infections in control group.
During and at end of exposure, determine prevalence of patent virus infections in exposed group.

FIGURE 1. Scheme for stepwise approach to use of *Baculovirus*-shrimp system in combination as a bioassay to detect possible interactions among pollutant (Aroclor 1254), virus, and host.

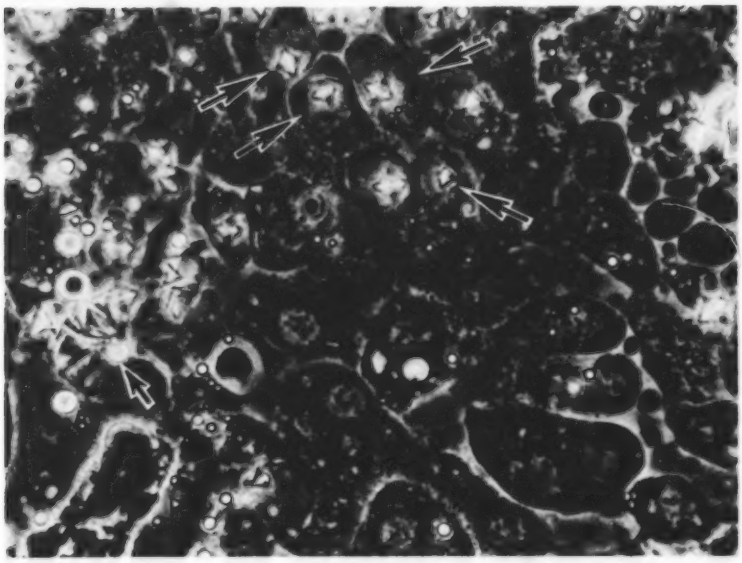

FIGURE 2. Patent *Baculovirus* infections of shrimp hepatopancreatic cell nuclei; note polyhedral inclusion bodies (PIBs) in hypertrophied nuclei (arrows) and PIBs liberated from lysed cells (phase-contrast microscopy).

hepatopancreata and 15 tails (muscular portion) from exposed shrimp were analyzed for the presence of Aroclor. Weekly samples of 35 shrimp were taken from the exposure tank during the exposure experiment, and the prevalence of patent virus infections in each of the samples was determined. Laboratory microscopic examination of shrimp for patent virus infections has been described by Couch.[6] The relative intensity of individual patent infections was recorded for each sample as light, moderate, or heavy. Mortality of shrimp was monitored daily during the experiment, and a count of surviving shrimp was made when the experiment ended.

The control tank received seawater continuously from a siphon that supplied 360 liter/hr, the same as that supplied to the exposure tank. The solvent carrier triethylene glycol, without PCB, was injected into the control tank water at a rate identical to that for the exposure tank. During the experiment, water and shrimp samples were taken from the control tank, on the same schedule as that of exposed samples, for gas chromatography and viral prevalence and infection analyses. Shrimp mortality in the control tank was monitored and recorded daily, and survivors were counted and examined for the presence of virus when the experiment ended.

Experimental and control viral frequency data were plotted as prevalence of virus per weekly sample for 5 weeks. Least-squares and joint confidence interval regression analyses were used to determine significant differences in the slopes of the plotted prevalence data.

FIGURE 1 shows a general scheme of the steps involved in the experimental-control procedure for evaluating possible enhancement of viral frequency in shrimp exposed to chemical stressors (pollutants).

RESULTS

Virus-Related Cytopathology

Cytopathologic effects of the shrimp *Baculovirus* have been described in detail by Couch[5,6,8] and Couch *et al.*[7] Patent infections in naturally exposed shrimp have been determined, via light microscopy, by the presence of from one to several characteristic polyhedral (tetrahedral) inclusion bodies (PIBs) in hypertrophied hepatopancreatic cell nuclei (FIGURE 2). Heavy prepatent infections can also be characterized by the presence of numerous hepatopancreatic cells with hypertrophied nuclei minus the formed PIB (FIGURE 3). The number of PIBs per cubic millimeter of hepatopancreas is used to determine the relative intensity of individual infections. More than 1100 PIBs/mm^3 of tissue is considered to be a relatively severe infection.

Uptake of Aroclor 1254 in Exposed Shrimp

Shrimp in the experimental tank water exposed to approximately 0.6–0.7 μg/liter of Aroclor 1254 for 35 days accumulated up to 21 mg/kg of Aroclor (35,000 × ambient level) in their hepatopancreata, the major site of viral infection (FIGURE 4). In contrast, there was a gradual, but much lower, accumulation of Aroclor in the tail muscle, a tissue not infected by the virus (FIGURE 4). Shrimp and water samples

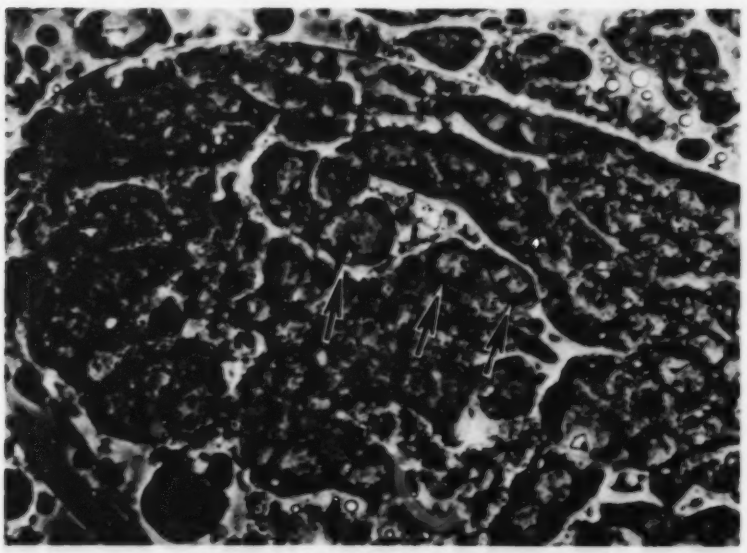

FIGURE 3. *Baculovirus*-infected nuclei (arrows) without PIBs (prior to PIB formation); note hypertrophied nuclei characteristic of this prepatent stage in the viral reproductive cycle (phase-contrast microscopy).

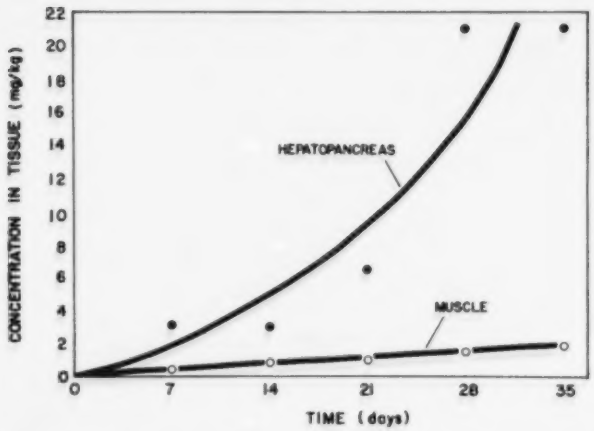

FIGURE 4. Graph of uptake and accumulation of Aroclor 1254 (PCB) in two tissues of pink shrimp over a continuous exposure period of 35 days. Ambient exposure concentration was 0.6–0.7 ppb of Aroclor in seawater.

taken weekly from the control tank and analyzed for the presence of Aroclor showed no trace of the compound.

Viral Prevalence and Mortality in Aroclor-Exposed Shrimp Versus Control Shrimp

The base sample of 150 shrimp, examined immediately prior to the beginning of the exposure experiment (time 0, FIGURE 5), revealed a base prevalence† of 23.3% of patently infected shrimp. All of these infections were light. Thereafter, a gradually greater increase in viral prevalence was found (upper line, FIGURE 5) in the Aroclor-exposed shrimp samples (chemically stressed) than in the control shrimp samples (lower line, FIGURE 5). The viral prevalence in the last sample of exposed shrimp was 75% after 35 days of exposure. The prevalence of virus in the last nonexposed control shrimp sample was 45.7% after 35 days of holding. The slopes of the lines in FIGURE 5 are significantly different ($p = 0.05$) according to forced regression analysis.

Mortality of shrimp in the Aroclor exposure tank was increasingly higher than that in the control tank. By 35 days, the cumulative mortality in the experimental tank, was 50%, whereas mortality was only 13% in the control tank at 35 days (FIGURE 5).

It is invalid to attempt to correlate the higher mortality of exposed shrimp with the higher viral prevalence in the chemically stressed shrimp, because Aroclor 1254 alone, even in low concentrations, is toxic to penaeid shrimp. Only naturally infected shrimp were available for this test; it was therefore impossible to use a positive chemical control (*known noninfected* shrimp exposed to Aroclor 1254) in the experiment. Ideally, one should have a noninfected source of shrimp for determining the

†Prevalence = number of patently infected shrimp in sample × 100/total number of shrimp in sample.

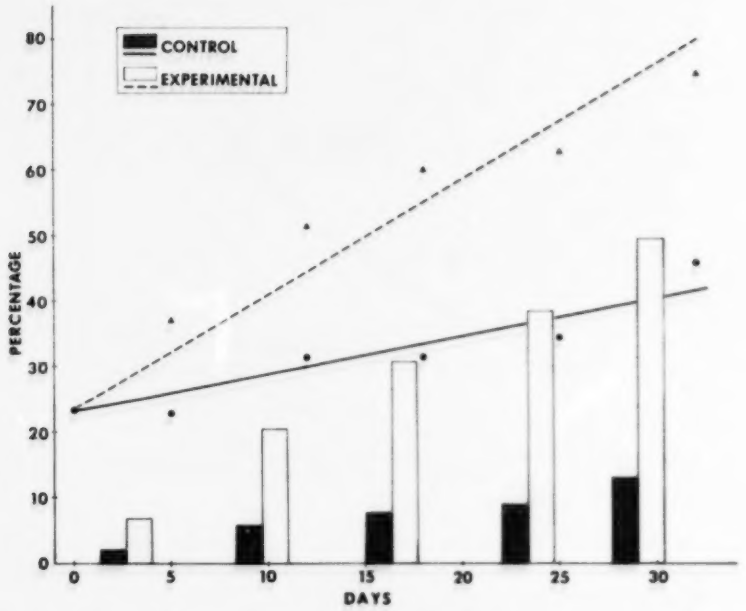

FIGURE 5. Graph of viral prevalence and mortality in Aroclor 1254-exposed shrimp samples and in control shrimp samples (original viral frequency in beginning exposed and control shrimp was 23.3%). Exposed shrimp had a greater and more rapid increase in viral prevalence and cumulative mortality than did controls (dark histogram, cumulative mortality of controls; light histogram, cumulative mortality of exposed).

effect of the chemical alone on mortality. At the present time, this requirement cannot be satisfied due to our inability to detect all latent viral infections or to rear shrimp xenobiologically.

DISCUSSION

The most significant finding of this study was that the chemically stressed (Aroclor exposed) shrimp samples had progressively higher prevalences of virus than did the control (nonexposed) shrimp samples. Both exposed and control groups used in the experiment had the same prevalence of patent viral infections at the beginning of the test (FIGURE 5). The rather abrupt increase in viral prevalence in the Aroclor-exposed shrimp (from 23.3 to 75% in 35 days) probably was related to an undefined interaction of host, chemical stressor (Aroclor 1254), and virus. The rather gradual rise in viral prevalence in the control shrimp (from 23.3 to 45.7% in 35 days) was within the range that we have observed in shrimp maintained in unusually crowded conditions in aquaria without chemical stress.‡

There are several possible interactions among the host, chemical stressor, and

‡The mechanism of viral transmission appears to be cannibalism and feeding of shrimp on fecal material.

virus that could account for the more rapid increase of viral infections in chemically stressed shrimp than in control shrimp. Among these possibilities are loss of resistance to new viral infections in shrimp hosts due to toxic effects of Aroclor 1254, enhancement of latent or occult viral infections possibly carried by all or most of the shrimp from enzootic viral populations, increase in virulence of virus when exposed *in vivo* to Aroclor 1254, and greater susceptibility of intoxicated, weakened individuals to the cannibalistic habits of lesser intoxicated individuals in the exposed group of shrimp.

We plan to use the simple *Baculovirus*-shrimp system (FIGURE 1) in future bioassays with a variety of chemicals that are aquatic ecosystem pollutants. The history and potential uses of the system to test the "host-pathogen-pollutant interaction" concept is outlined in FIGURE 6.

The tentative criteria for evidence of interaction are increase in viral prevalence in stressed versus control shrimp, increase in infection intensity in pollutant-exposed individuals as compared to controls, increase in exposed-infected shrimp mortality versus control shrimp mortality, and enhanced cytopathic effects in exposed-infected shrimp as compared to those in control-infected shrimp.

SUMMARY

A large group of shrimp, 23.3% of which had light patent *Baculovirus* infections, was divided equally into two groups. One group was exposed to the chemical

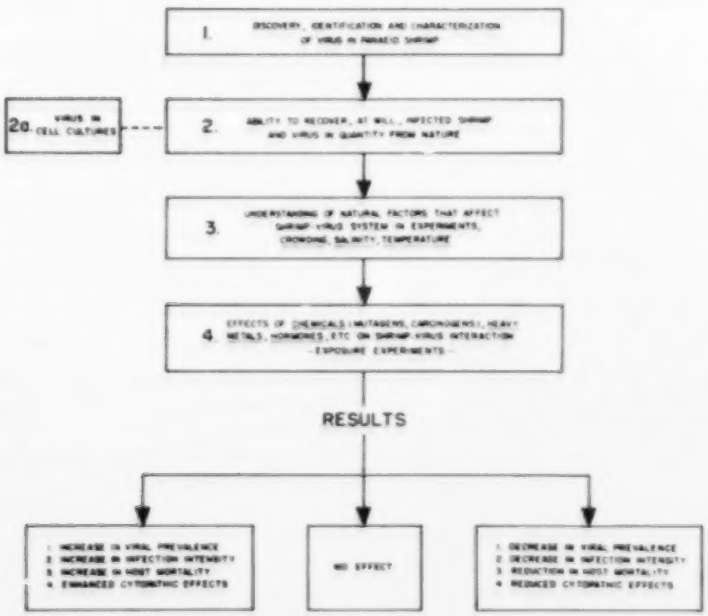

FIGURE 6. Schematic history and potential use of the shrimp-virus model for evaluating possible interactions (chronic sublethal effects) among pollutants, virus, and host.

stressor Aroclor 1254 (a polychlorinated biphenyl) at 0.7 ppb for 35 days in flowing seawater. The other group was maintained as a control group in flowing seawater. Viral prevalence in exposed shrimp samples increased with time at a significantly greater rate than did viral frequency in control shrimp. Viral prevalence in Aroclor-exposed shrimp survivors was 75% after 35 days, whereas in control shrimp, only 45.7% had patent viral infections. This finding suggests an interaction among chemical stressor (Aroclor 1254), host, and virus. The nature or mechanism of this interaction has not been defined, but the shrimp-virus system shows promise for future bioassays of influence of low concentrations of pollutants on natural pathogen-host interactions.

Acknowledgments

Mr. Lowell Bahner conducted the statistical tests, and Mr. Steve Foss aided in the preparation of Figures. The staff of the chemistry laboratory at Gulf Breeze Environmental Research Laboratory are thanked for analyses of Aroclor 1254 samples.

References

1. Snieszko, S. F. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. J. Fish Biol. **6:** 197–208.
2. Wedemeyer, G. 1970. The role of stress in disease resistance of fishes. *In* A Symposium on Diseases of Fishes and Shellfishes. S. F. Snieszko, Ed.: 30–35. American Fisheries Society. Washington, D.C.
3. Pippy, J. H. C. & G. M. Hare. 1969. Relationship of river pollution to bacterial infection in salmon (*Salmon salar*) and suckers (*Catastomus commersoni*). Trans. Amer. Fisheries Soc. **98:** 685–690.
4. Couch, J. A. & D. R. Nimmo. 1974. Detection of interactions between natural pathogens and pollutants in aquatic animals. *In* Proceedings of the Gulf Coast Regional Symposium on Diseases of Aquatic Animals. R. L. Amborski, M. A. Hood & R. R. Miller, Eds.: 261–268. Louisiana State University Press. Baton Rouge, La.
5. Couch, J. A. 1974. Free and occluded virus, similar to *Baculovirus*, in hepatopancreas of pink shrimp. Nature (London) **247:** 227–231.
6. Couch, J. A. 1974. An enzootic nuclear polyhedrosis virus of pink shrimp: ultrastructure, prevalence, enhancement. J. Invert. Pathol. **24:** 311–331.
7. Couch, J. A., M. D. Summers & L. Courtney. 1975. Environmental significance of *Baculovirus* infections in estuarine and marine shrimp. Ann. N.Y. Acad. Sci. **266:** 528–536.
8. Couch, J. A. 1976. Attempts to increase *Baculovirus* prevalence in shrimp by chemical exposure. Progr. Exp. Tumor Res. **20:** 304–314.
9. Nimmo, D. R., D. J. Hansen, J. A. Couch, N. R. Cooley, P. R. Parrish & J. I. Lowe. 1975. Toxicity of Aroclor® 1254 and its physiological activity in several estuarine organisms. Arch. Environ. Contam. Toxicol. **3:** 22–39.